

# Bovine Mastitis

## The Relation of Streptococci to Physical Changes Occurring in the Udders of Dairy Cows

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THE anatomical changes which develop in the bovine udder as a result of mastitis offer one of the most satisfactory means available at the present time of determining whether the organ is diseased and how far the disease has progressed. When mastitis has been present in a quarter for even a relatively short time the character of the udder tissue becomes appreciably altered. The changes which are found on clinical examination of a diseased quarter may be either in the form of distinct firm nodules or diffuse thickening of the glandular tissue. The lesion in either case represents an invasion of the normal secreting tissue by connective or scar tissue following inflammation of the gland. If the mastitis is only transient in nature, the proliferative process becomes static, but the indurations remain as a permanent record of the attack. Unfortunately, the majority of the cases of mastitis are caused by bacterial invasion, and as a rule the infection establishes itself permanently in the udder. In this event, the disease tends to progress with varying degrees of rapidity from the original site of localization, usually the cistern, to the rest of the quarter. Induration of the glandular tissue accompanies this progression until in

many cases there is practically a complete replacement of the secreting tissue by scar tissue.

In view of the permanence of the changes which occur as a result of mastitis and its accessibility for examination, palpation of the empty udder affords an easy and accurate means of detection of the disease. Some of the possibilities inherent in this method have been recognized in the past by a number of authors<sup>15</sup> and Udall and Johnson<sup>20</sup> have observed that "the value of a physical examination in diagnosis (of mastitis) rates above any other single method." That the milk from udders which are definitely indurated is usually changed in composition as shown by other tests; such as pH, chlorine, catalase and leucocyte count, has been observed in the course of mastitis investigations in this laboratory and this fact has also been reported by Hucker and Udall.<sup>10</sup> On the other hand, when milk samples from these udders are examined bacteriologically, bacteria which are not customarily associated with mastitis are not infrequently found. Also a few udders have been encountered which appear to be entirely normal on palpation and are harboring mastitis streptococci.

As a result of the rather extensive investigation of mastitis by a number of workers in the past, a number of species of bacteria have been described as the causative agents of the disease. It is fairly well agreed at the present time, however, that the streptococci are of chief importance in this respect and that one definite species, *Streptococcus agalactiae*, is found in a high percentage of the cases of this type of mastitis. The incidence frequently reported for this species has been around 90 per cent.<sup>8, 17</sup> Several species of streptococci comprise the remaining 10 per cent, but probably because of their relatively infrequent occurrence, the information concerning them is somewhat limited. Other species of cocci, more particularly staphylococci are also found in some cases of mastitis, but comparatively little knowledge is available relative to their importance in the disease. More recently however, reports<sup>5, 6, 11, 12, 18</sup> have appeared which indicate an increasing interest in this phase of the problem. The remaining bacterial species, consisting principally of bacilli, such as coliform types, *Corynebacterium pyogenes*, and others, are of minor importance probably because of the sporadic character of their occurrence. It is worthy of note in this connection that the changes induced by these various agents in the udder and the milk are very similar.

The object of this work therefore was to compare the physical condition of the udders of infected and uninfected cows in herds in which mastitis streptococci were present and also to compare the udders of cows in streptococcus-free herds with these animals. In addition an attempt was made to identify the species of streptococcus in each animal so infected. Several different types of herds in the vicinity of Beltsville, Md., were included in the investigation which was started in the fall of 1936. Three of these herds,

numbers 1, 2, and 9 (Tables II and V) are maintained to supply fluid milk to institutions. Two other herds, numbers 3 and 5, are used principally for experimentation in breeding and feeding problems of dairy cattle. The remaining four herds, numbers 4, 6, 7, and 8, are privately owned and are subject to health department supervision. In addition to milk production, herd number 4 is used for breeding pure bred cattle.

#### PROCEDURE

Composite samples of milk were collected from each animal immediately before milking. The ends of the teats were washed thoroughly with a pledget of cotton, moistened with a 1 in 1,000 solution of bichloride of mercury in 50 per cent alcohol. Several streams of milk were discarded from each quarter and then as equal amounts as possible were drawn from the four quarters into a sterile screw top specimen vial, holding about 20 c.c. When the samples were brought to the laboratory, part of the milk was immediately tested by the Hotis method,<sup>9</sup> and the remainder was incubated for 24 hours at 37° C. in the original container. At the end of this time, the results of the Hotis test were noted and smears made from the incubated milk for microscopic examination. In addition a large loopful (4 mm. loop) of incubated milk was spread over the surface of a blood agar plate (5 per cent horse blood) which had been prepared previously. The plates were incubated for 24 hours at 37° C., and the colonies which resembled streptococci were picked into 10 per cent serum (horse) bouillon. These cultures were examined microscopically for purity following incubation and then seeded in differential culture media.

One-tenth c.c. of culture was added to each of the following media: litmus milk, methylene blue milk (1 in

10,000), lactose, salicin, mannite, trehalose, sorbitol, aesculin, and sodium hippurate. The media for the fermentation tests were made by adding 1 per cent of the carbohydrate to extract broth together with 10 per cent serum (horse) and Andrade's indicator. The solution was adjusted to pH 7.4, sterilized by filtration, and tubed in 2 c.c. amounts. Each medium was incubated for 48 hours at 37° C., to test for sterility. The sodium hippurate medium was prepared according to the pepsin-peptone formula recommended by Ayers and Rupp.<sup>1</sup> Ability to split aesculin was determined by the method described by Weatherall and Dible.<sup>21</sup> The reactions of the streptococci in these test substances were recorded after 5 days' incubation at 37° C. All of those cultures which failed to give typical reactions for *S. agalactiae* (group A, Table I) were tested a

second time after an interval of several months as a check on the results noted at the time of isolation.

Each udder was examined clinically as soon as possible after it had been milked out. Classification of the udders follows the plan described by Udall and Johnson<sup>20</sup> in so far as the physical condition of the organ is concerned. The brom-thymol-blue test was not used and the previous history of the animal was not considered. In this classification, a cow is placed in Class 1 when all quarters are entirely normal and free of indurations. When one or more quarters have some slight change in the consistence of the glandular tissue which may be in the form of a minimum diffuse or nodular thickening, usually in the lower part of the gland, the cow is placed in Class 2. If one or more quarters of the udder contain distinct indurated

TABLE I

*Types of Streptococci Isolated from Milk Samples Collected in 5 Streptococcus-infected Herds*

Culture	Litmus Milk			M.B. Milk *		Lactose	Salicin	Mannite	Aesculin	Sodium Hippurate	Herd Number					Total Cultures
	A	C	R	C	R						1	2	3	4	5	
	Trehalose +										Sorbitol —					
A	+	+	<u>P</u> or	—	—	+	<u>+</u> or	—	—	+	21	105	41	24	0	191
B	+	—	—	—	+	+	—	+	+	+	1	1	0	0	0	2
C	+	+	P	+	+	+	+	+	+	<u>+</u> or	3	2	0	0	0	5
				Trehalose +					Sorbitol +							
D	+	+	<u>S</u> or	+	+	+	+	+	+	+	16	1	2	0	0	19
E	+	<u>+</u> or	<u>+</u> or	<u>+</u> or	<u>+</u> or	+	+	<u>+</u> or	—	+	6	2	1	4	1	14
F	+	—	—	<u>+</u> or	<u>P</u> or	+	—	—	—	+	6	0	0	0	0	6
				Trehalose —					Sorbitol —							
G	+	+	<u>S</u> or	—	—	+	+	<u>S</u> or	—	+	32	0	0	0	7	39

\* Methylene blue milk  
+ = Positive

A = Acid  
P = Partial

R = Reduction  
S = Slight

C = Coagulation  
— = Negative

areas either diffuse or as nodules, but with a considerable part of the glandular tissue remaining intact, the cow is classed as number 3. A cow is considered to be in Class 4 when a major portion of the secreting tissue has been replaced by connective or scar tissue.

#### RESULTS

A total of 629 lactating cows in 9 dairy herds have been examined in the course of this investigation. Streptococcic mastitis was found in 5 herds, containing 547 animals (Table II) while no streptococci were detected in the remaining 4 herds with 82 cows (Table V). The number of cows found to be excreting streptococci with the milk by one or more of the tests was 298, or 47.4 per cent of all animals examined. This result is more or less comparable to those reported by other authors.<sup>15</sup> The highest incidence of infection occurred in the largest herds, numbers 1 and 2, with percentages of 63.1 and 61.0 respectively (Table II), while the lowest percentage, aside from herds which were completely free and which might be considered in another category, was 32.0 in herd 5 with only 25 animals. Herds 3 and 4 had been found to have a somewhat larger num-

ber of animals infected at the time of previous examinations, but owing to disposal of older animals and their replacement with heifers, the incidence of infection has been materially reduced, particularly in Herd 4.

Of the 298 samples (Table II) in which streptococci were detected by one or more of the tests, cultures were recovered from 276, or 90.2 per cent. The different types of streptococci which were isolated have been divided into three large groups (Table I) on the basis of their action on sorbitol and trehalose<sup>2</sup> and these groups further sub-divided with respect to the results in the other media employed. As a consequence, 7 more or less distinct types have been formed. Because of certain differences in the composition and kinds of media used, it is somewhat difficult to compare the results of other authors in connection with the bacteriology of the streptococci. However, there can be relatively little doubt that Group A in Table I is *S. agalactiae* which has been described under that name by some authors<sup>7, 19</sup> and Group 1 A<sup>16</sup> and Group 1<sup>4</sup> by others. On the other hand, the similarity of the remaining strains found in this work with those reported elsewhere is not as

TABLE II

*Incidence of Streptococcus Agalactiae and Other Types of Streptococci in 5 Dairy Herds*

Herd Number	Number of Milking Cows in Herd	Number of Streptococcus Infected Cows	Per cent of Streptococcus Infected Cows	Number of Cows Carrying <i>S. agalactiae</i> <sup>6</sup>	Per cent of Cows Carrying <i>S. agalactiae</i>	Number of Cows Carrying Other Types of Streptococci <sup>4</sup>	Per Cent of Infected Cows Carrying Other Types of Streptococci
1	141	89 <sup>1</sup>	63.1	53	62.3	32	37.7
2	190	116 <sup>2</sup>	61.0	105	94.4	6	5.6
3	88	44	50.0	41	93.2	3	6.8
4 <sup>6</sup>	103	41 <sup>3</sup>	39.8	24	85.7	4	14.3
5	25	8	32.0	7	87.5	1	12.5
Totals	547	298	54.5	230	82.6	46	16.9

1. Streptococci were not isolated from 4 samples of milk.

2. " " " " " 5 " " " " " .

3. " " " " " 13 " " " " " .

4. 7 strains of this group typical of *S. agalactiae* other than fermentation of both trehalose and sorbitol.

5. 39 strains failed to ferment trehalose.

6. Large percentage of first-calf heifers.

clear. As far as can be determined, Groups B, C, and F have not been reported in other publications dealing with this subject. Group D appears similar in many respects to *S. uberis* described by Diernhofer<sup>3</sup> which is listed as Group 3 in the classification of Plastring and his associates.<sup>16</sup> Group E likewise may be said to resemble Group III of Edwards.<sup>4</sup> Failure by Group G strains to acidify trehalose is the only cultural difference between them and Group A. Inasmuch as approximately half of the cultures in Group G showed definite evidence of fermentation of trehalose when tested a second time several months after isolation, some question might be raised concerning their inclusion in a separate group rather than placing them in Group A. The irregularity of trehalose fermentation has also been noted by Plastring *et al.*<sup>16</sup> who have, however, given similar strains a separate classification. It will be observed in Table I that Group G strains have been found in but 2 of the 5 infected herds, numbers 1 and 5 (Table I). In the former, 56.1 per cent of the streptococcus cultures isolated belonged in this group, whereas in the latter herd all but one animal were found to be carrying this strain. In Table II, animals carrying Group G streptococci have been included with those carrying typical *S. agalactiae* or Group A types for convenience in tabulation.

The results of physical examination

of the udders of 617 cows appear in Table III. Twelve additional animals were not examined because of too recent freshening and an accompanying edema of the udder which rendered the organ unsuitable for palpation. The figures presented in Table III are instructive with respect to the influence of streptococci on the udder and also as to the condition of the udders of cows which are not so infected. Although the total number of animals in each group is approximately equal, 2.4 per cent of the infected animals had udders in which no indurations were found and were placed in Class 1 while 11.6 per cent of the uninfected animals fell in this class, a difference of almost 5 to 1. There were 18.2 per cent of the infected animals in Class 2 and 29.4 per cent of uninfected animals which is a ratio of not quite 2 to 1. No significant difference was apparent between the percentage of animals in the two groups found to be in Class 3, although a slight advantage was noted for the uninfected group. In Class 4, however, the difference is considerable in that 24.7 per cent of the infected cows showed marked physical evidence of mastitis in one or more quarters as contrasted with 9.2 per cent for the uninfected group, or about two and one-half times as many. The fact that the percentage of cows in the two groups not infected with streptococci is nearly the same in all 4 classes would appear to emphasize the damaging

TABLE III

Summary of Average Results of Classification of Udders of Cows in the Several Herds by Physical Examination

Group	Total Number of Cows	Class 1		Class 2		Class 3		Class 4	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Streptococcus-infected cows	291	7	2.4	53	18.2	159	54.6	72	24.7
Uninfected cows in all herds	326	38	11.6	96	29.4	162	49.7	30	9.2
Uninfected cows in streptococcus-infected herds	244	28	11.5	74	30.3	120	49.1	22	9.0
Cows in streptococcus-free herds	82	10	12.3	22	26.8	42	51.2	8	9.7
All cows examined	617	45	7.3	149	24.1	321	52.0	102	16.5

TABLE IV

*Classification of Udders of Cows in Streptococcus-Infected Herds by Physical Examination*

Herd Number	Number of Milking Cows in Herd	Class 1		Class 2		Class 3		Class 4	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
1	136 <sup>1</sup>	8	5.9	31	22.8	81	59.5	16	11.7
2	183 <sup>2</sup>	17	9.3	33	18.0	89	48.7	44	24.0
3	88	6	6.8	26	29.5	39	44.3	17	19.3
4	103	2	1.9	31	30.0	55	53.4	15	14.5
5	25	3	12.0	5	20.0	16	64.0	1	4.0
Totals	535	36	6.7	126	23.5	280	52.3	93	17.4

1. Five additional cows not palpated

2. Seven additional cows not palpated

effect of these organisms and may also possibly indicate some other common cause for the fibrosis present in non-streptococcus udders.

The physical condition of the udders found in the different herds is shown in Tables IV and V. Although there is considerable variation in this respect in both groups, it will be noted that when individual herds are considered, the uninfected ones do not show the amount of change in the udders found in those which are affected with streptococcic mastitis. It should also be pointed out that the largest number of Class 4 cows occurred in Herd 2 which was also found to have the highest incidence of infection with *S. agalactiae*, 94.4 per cent (Table II), and that as the number of animals carrying these streptococci decreases in the first 4 herds, the number of cows in Class 4 becomes smaller. Under the circumstances, it would appear that this result, although interesting, may be more or less co-

incidental since the percentages in the other classes do not support it.

Because of the comparatively small number of animals in the 4 herds in which streptococci were not found, considerable fluctuation appears in the individual herd percentages. Inasmuch as no definite cause could be determined for the changes present in these herds, it may be possible that local conditions were of considerable influence in this matter. In Herd 9, as an example, several animals with udders showing marked evidence of mastitis and also carrying streptococci had been eliminated several years prior to this survey and replacements made with first-calf heifers from the herd. However, the percentages for these herds are in fairly close agreement with the uninfected cows (Group B, Table VI) in the infected herds. In Table VI, the physical condition of the udders in the infected (A) and uninfected (B) groups in the same herd are compared.

TABLE V

*Classification of Udders of Cows in Streptococcus-free Herds by Physical Examination*

Herd Number	Number of Milking Cows in Herd	Class 1		Class 2		Class 3		Class 4	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
6	17	2	11.8	5	29.4	9	52.9	1	5.9
7	34	4	11.8	11	32.4	14	41.2	5	14.6
8	8	0	0.0	2	25.0	5	62.5	1	12.5
9	23	4	17.4	4	17.4	14	60.8	1	4.4
Totals	82	10	12.3	22	26.8	42	51.2	8	9.7

TABLE VI

*Comparative Results of the Classification of Udders of Infected\* and Uninfected Cows in Streptococcus-infected Herds*

Herd Number	Group	Total No. of Cows	Class 1		Class 2		Class 3		Class 4	
			No. of Cows	Per cent	No. of Cows	Per cent	No. of Cows	Per cent	No. of Cows	Per cent
1	A	86 <sup>1</sup>	2	2.3	17	19.8	54	62.8	13	15.6
	B	50 <sup>2</sup>	6	12.0	14	28.0	27	54.0	3	6.0
2	A	112 <sup>3</sup>	4	3.6	18	16.1	53	47.3	37	33.0
	B	71 <sup>4</sup>	7	16.9	16	22.7	35	49.2	8	11.2
3	A	44	1	2.3	11	25.0	20	45.4	12	27.2
	B	44	5	11.5	15	34.0	19	43.1	5	11.5
4	A	41	0	0.0	6	14.8	25	60.9	10	24.3
	B	62	2	3.2	25	40.3	30	48.4	5	8.1
5	A	8	0	0.0	1	12.5	7	87.0	0	0.0
	B	17	3	17.6	4	23.5	9	52.9	1	5.9

A = Infected animals

B = Uninfected animals

\* Cows included from which streptococci were not isolated

1. 3 additional cows not palpated

3. 4 additional cows not palpated.

2. 2 additional cows not palpated

4. 3 additional cows not palpated

Since all of the animals in each herd are maintained under the same conditions the results in this table are of considerable significance. The percentage of uninfected cows in Classes 1 and 2 is uniformly higher by a large margin, while the number of streptococcus-infected cows in Class 4 is two to three times that of the uninfected animals. It may be possible that the somewhat closer agreement between the uninfected animals in the different herds with regard to class percentage may be attributed to the larger numbers in each herd.

#### DISCUSSION

The results of the bacteriological study of milk samples in this investigation agree fairly well with the reports of other workers.<sup>15</sup> Figures somewhat higher than the 47.4 per cent infection with streptococci have been found in some cases while in others the percentage has been lower. Also the number of cows, 83.3 per cent carrying *S. agalactiae* in one or more quarters

approaches the previously mentioned figure of 90.0 per cent. This slightly lower percentage is due to the findings in one herd (number 1, Table II). If this herd is not considered, the percentage becomes 92.6. A number of types of streptococci were encountered in Herd 1, Table I, most of which have been described by other authors. The type of streptococcus most prevalent in this herd, however, resembled *S. agalactiae* in all respects with the exception of its failure to act on trehalose (Group G, Table I). This same variety was also practically the only one present in Herd 5 (Table I). Although it seems probable that these streptococci may be *S. agalactiae* or closely related to it, it is of interest to note that the percentage of cows with Class 4 udders in these herds is appreciably smaller than in those herds having a high percentage of cows infected with typical *S. agalactiae*.

Very few figures are available with which the results of the clinical examination of the udder can be directly

compared. It is therefore not known whether the figures of 7.3 per cent, Class 1, or normal udders; 24.1 per cent, Class 2, or slightly indurated udders; 52 per cent, Class 3, or distinctly indurated udders; and 16.5 per cent, Class 4, or udders with marked induration would be approximated in an examination of an equal number of cows elsewhere. In connection with the percentage of Class 4 animals, however, some figures reported by the Bureau of Animal Industry<sup>13, 14</sup> several years ago are of interest. At this time 235,354 milk cows were examined for marked physical evidence of mastitis, and 28,439, or 12.0 per cent, were removed on this account, while 4,760 additional cows were regarded as being suspicious. In the milk shed of one of the larger cites, 64,041 cows were examined and 9,027, or 14.1 per cent, were found to have marked physical cases of mastitis. In view of these results it would appear that the percentage of cows in Class 4 found here could hardly be considered abnormally high. If a similar agreement should extend to the other three classifications, then it might be said that roughly 10 per cent of the cows in milk production have normal udders, 25 per cent have reasonably good udders, while 50 per cent show definite evidence of mastitis, and finally 15 per cent or 1 out of every 6.5 animals are no longer suitable for dairy purposes.

That the streptococci are responsible for the larger portion of the indurations found in these udders seems to be clearly demonstrated by this work, but it is also equally evident that even if the streptococci were eliminated a certain amount of mastitis would still be present in the herd. These same facts are also of importance with regard to the value of clinical examination of the udder in the detection of mastitis. Cows which have or have had mastitis, irrespective of the cause, for any ap-

preciable length of time can be detected by this method with remarkable consistency, and in addition this method is extremely useful in selecting animals which should be removed from the herd on account of badly diseased udders.

#### SUMMARY

Milk samples from 629 cows in 9 dairy herds were examined by 3 bacteriological methods for the presence of streptococci. The 3 methods used were the Hotis test, microscopic examination of incubated milk, and culture of incubated milk on blood agar plates. When streptococci were found on the blood agar plates, they were tested in differential media and placed in groups according to their action on these media.

Streptococci were detected in milk samples from 298, or 47.4 per cent of all animals examined. These animals, however, were present in only 5 herds. No streptococcus infected cows were found in 4 herds with a total of 82 animals. The highest incidence of infection occurred in the largest herds, and the lowest in the smallest of the 5 herds.

Although streptococci were detected in 298 milk samples by the 3 tests, cultures were recovered from 276, or 90.2 per cent. From their action on trehalose and sorbitol, these strains were divided into 3 large groups and further divided into sub-groups on the results on the other media. Seven groups were found. Group A, or *S. agalactiae*, predominated, and when Group G, which differed from it only in the latter's failure to acidify trehalose, is included, formed 83.3 per cent of the cultures isolated. About one-half of the Group G strains fermented trehalose when tested a second time several months later.

The udders of 617 cows were examined by palpation for indurations as



soon as possible after they were milked out. On the basis of physical condition 45, or 7.3 per cent, of the animals were placed in Class 1, or free of indurations; 149, or 24.1 per cent, were in Class 2 with slight indurative changes; 321, or 52.0 per cent, were definitely indurated and placed in Class 3; and 102, or 16.5 per cent, showed marked physical evidence of mastitis, and belonged in Class 4.

There were 291 cows infected with streptococci as compared with 326 in which these organisms could not be detected. Of the infected animals 7, or 2.4 per cent, of the udders were in Class 1, while 38, or 11.6 per cent, of the uninfected cows fell in this group; a difference of about 5 to 1. In Class 2 there were 53, or 18.2 per cent, of infected cows and 96, or 29.4 per cent, of uninfected cows, a ratio of not quite 2 to 1. There was no significant difference in Class 3 between the two groups, but in Class 4 there were 72, or 24.7 per cent, infected cows against 30, or 9.2 per cent, uninfected, or about two and one-half times as many as the former.

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